destroying waste materials. One example of this is lipase, which is used for digesting waste onboard ships. The enzymes are genetically engineered to include a poly-His tail as well as other stabilizing amino acid substitutions. As used herein, the term "amino acid substitution" includes both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues. A "stabilizing amino acid substitution" is a substitution that non-covalently bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme. Non-covalent enzyme immobilization on polymerized liposomes was effected by co-polymerizing amphiphiles containing metal salts of iminodiacetic acid or nitrilotriacetic acid with other polymerizable amphiphiles and then binding the enzyme to the iminodiacetic acid-metals or NTA-metal salts on the outer surfaces of the vesicles. This technique relies on the strong binding affinity between iminodiacetate salts or NTA salts and polyhistidine, which has been made available on the surface of the enzyme selected for immobilization through genetic engineering. The enzymes that can be used for this technique are those enzymes that have appropriately reactive surface available histidines or which have a histidine tag that can be added through site specific mutagenesis [metagenesis]. This includes, or course, polyhistidine. Histidine forms a strong bond with iminodiacetic acid salts, such as copper, zinc, cobalt, and nickel iminodiacetate salts, and nitrilotriacetic acid salts, such as copper, zinc, cobalt, and nickel salts. The main criterion for this process to be effective is that the binding site on the enzyme be far away from or innocuous to the function of the enzyme's catalytic site. While silica is the preferred inorganic surface because it is relatively inexpensive and its properties are well understood, any type of metal oxide ceramic particles that can be formed similar to the Stober process starting with a metal alkoxide precursor can be used. Other types of inorganic surfaces that can be used in the process of the present invention include alumina, baria, titania, and zirconia.

sub B1 cont

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Paragraph beginning at page 6, line 20

Bachmair et al., in U.S. Patents 5,646,017; 5,496,721; 5,196,321; 5,132,213; and 5,093,242, the entire contents of which are hereby incorporated by reference, disclose methods for designing or modifying protein structure at the protein or genetic level to produce proteins having specified amino-termini in vivo or in vitro. These methods can be used to produce proteins having amino-termini on enzymes wherein gene encoding the enzymes can be made to encode an amino acid of the desired class at the amino-terminus so that the expressed enzyme exhibits a predetermined amino-terminal structure which renders it metabolically stable and able to bind to metal salts of iminodiacetic acid which are copolymerized with amphiphiles. Preferably, the amino-terminal structure is histidine, although C-terminal or internal poly-His sequences will usually be satisfactory as well.

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Paragraph beginning at page 7, line 15

The enzymes useful in detoxifying nerve agents are attached to iminodiacetate salt groups on the surface of silica particles formed by co-hydrolyzing TMOS with an IDA-alkoxysilane derivative. The IDA-alkoxysilane derivative accounted for 5 weight percent of the total silica content. After particles were synthesized using the Stober procedure, the copper salt of the surface IDA groups was formed by adding an aliquot of 20% aqueous $CuSO_4$ solution (wt/wt) to the dry particles, and then suspending the particles using mild sonication or vortex mixing. The suspension was centrifuged and the supernatant was removed. This procedure was repeated, and the resulting blue silica particles were washed with water by adding the water to the particles, suspending the particles in solution, and then centrifuging the suspension and removing the supernatant. This procedure was repeated three times. Then, an aliquot of the thioesterase in 0.05 M phosphate buffer, pH 7.2, was added to a suspension of the particles in the same buffer. The suspension was incubated at 4°C for three hours. The particles were then centrifuged and the supernatant was removed. The particles were then washed using the phosphate buffer described above. All operations involving the enzyme were performed at 4°C. After the final

a4

a4 cont washing, the particles were resuspended in the buffer and stored for future use. The activity of the immobilized enzyme was confirmed using standard procedures.

Paragraph beginning at page 8, line 13

The gene for thioesterase-1 (TE-1) of E. coli strain JM109 was cloned using a modification of the procedure published in Escherichia coli: thioesterase I. Molecular cloning and sequencing the structural gene and identification of a periplastic enzyme, Hyeson Cho, John L. Carona (1993) Journal of Biological Chemistry 268: 9238-9245. Briefly, amplified DNA encoding the TE-1 protein and appropriate flanking nucleotide sequences was ligated into the DNA vector PCR 2.1 (Invitrogen). After preparing of 140 micrograms of the PCR2.1-TE1 vector DNA from 100 ml overnight culture, the engineered TE-1 fragment was liberated from the intermediate vector by digestion of 10 micrograms of this DNA with 20 units each of the restriction endonucleases NdeI and XhoI at 37°C overnight. The liberated TE-1 coding fragment was purified electrophoretically on a 2% agarose gel. The stained gene fragment was excised from the gel and subsequently obtained free of agarose using commercial products (Qiagen).

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Paragraph beginning at page 9, line 3

The gene for N-terminal polyhistidine-modified TE-1 was prepared by enzymatically ligating approximately 300 μg of the gene fragment described above with about 100 ng of pProEx-1 vector DNA (Life Technologies) previously digested with NdeI and XhoI enzymes and dephosphorylated with calf intestinal alkaline phosphatase. Transformed E. coli DH5αF'LacI^q cells (Life Technologies) were screened for the presence of the TE-1 inserted gene by electrophoretic analysis of differential whole-cell protein profiles of cells taken from small scale cultures grown plus and minus 1 mM isopropylthiogalactopyranoside (IPTG) chemical inducer.

a6

Please amend the abstract as follows.